DATA EVALUATION RECORD
EARTHWORM SUBCHRONIC TOXICITY TEST
OPPTS 850.6200

1. **CHEMICAL:** Novaluron PC Code No.: 124002

2. **TEST MATERIAL:** Chlorophenyl Urea Purity: 99.3%

3. **CITATION:**

Author: Rodgers, M.H.

Title: Chlorophenyl Urea Acute Toxicity (LC_{50}) to the Earthworm *(Eisenia foetida)*

Study Completion Date: ~~36941~~ Feb. 20, 2003

Laboratory: Huntingdon Life Sciences Ltd.
P.O. Box 2, Huntingdon,
Cambridgeshire, PE18 6ES, England

Sponsor: Makhteshim Chemical Works Ltd.
P.O. Box 60
Beer Sheva 84100, Israel

Laboratory Report ID: MAK 619/003779;
R-11872 (Sponsor Number)

MRID No.: 45638225

DP Barcode: D285479

4. **REVIEWED BY:** Rebecca Bryan, Staff Scientist, Dynamac Corporation

Signature: Rebecca Bryan **Date:** 4/1/03

APPROVED BY: Dana Worcester, Staff Scientist, Dynamac Corporation

Signature: Dana Worcester **Date:** 4/1/03

5. **APPROVED BY:** Bill Evans

Signature: William Evans **Date:** 11/26/03

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6. STUDY PARAMETERS:

Scientific Name of Test Organism: *Eisenia foetida*

Age/Size of Test Organism: Age not specified, 365-458 mg

Type of Test Concentration: Nominal

Definitive Study Duration: 14 days

7. CONCLUSIONS:

The earthworm, *Eisenia fetida*, was exposed to Chlorophenyl Urea, at nominal test concentrations of 95, 171, 309, 556, and 1000 mg/kg. By 14 days, there was 2.5, 88, and 100% mortality in the 309, 556, and 1000 mg/kg treatment groups, respectively. No mortality occurred in the control or 95 and 171 mg/kg treatment groups. Reductions in body weight by day 14 were 11 and 28% in the 556 and 1000 mg/kg treatment groups, respectively. **The LC_{50} was 447 mg/kg and the NOEC was estimated to be 171 mg/kg based on mortality and reductions in body weight.** This study is classified as Supplemental, because US EPA does not presently require subchronic toxicity testing with earthworms for pesticide registration, so SEP guidelines do not exist. The results of this study, however, are useful for risk assessment purposes.

Results Synopsis:

LC_{50}: 447 mg/kg 95% C.I.:407-485 mg/kg
NOEC:171 mg/kg Probit Slope: 12.2 (8.4-16.0)
LOEC: 309 mg/kg

8. ADEQUACY OF THE STUDY:

A. Classification: Supplemental

B. Rationale: US EPA does not presently require subchronic toxicity testing with earthworms for pesticide registration, so SEP guidelines do not exist. OPPTS guidelines exist for subchronic toxicity testing with earthworms and there were several deviations from these experimental protocol in this study.

C. Repairability: None. The results of this study are useful for risk assessment purposes.

9. GUIDELINE DEVIATIONS: This study was based on procedures of the OECD Guideline No. 207, "Earthworm, acute toxicity test", and ECC Directive 87/302/EEC, Part C, Methods for determination of ecotoxicity. Toxicity for earthworms, Artificial soil test.

1. The study duration was 14 days. Under the Ecological Effects Test Guidelines, "The test duration is 28 days" (OPPTS 850.6200, Earthworm Subchronic Toxicity Test, US EPA, Prevention, Pesticides and Toxic Substances (7104), EPA 712-C-96-167, April 1996, p.4, item 3(x)).

2. The weight of wet soil per replicate was 738 g. Guideline regulations specify that the wet soil weight per replicate shall be 270 g (OPPTS 850.6200, Earthworm Subchronic Toxicity Test, US EPA, Prevention, Pesticides and Toxic Substances (7104), EPA 712-C-96-167, April 1996, p. 7, Medium preparation, item (A))

3. The test chambers for this study were 1 liter glass containers. Guideline regulations specify that the tests chambers should be of a 1 pint capacity (OPPTS 850.6200, Earthworm Subchronic Toxicity Test, US EPA, Prevention, Pesticides and Toxic Substances (7104), EPA 712-C-96-167, April 1996, p. 7, Test chambers, item (A)).

4. A temperature range and pH (initiation) were reported for this study. Guideline regulations specify that temperature and pH measurements are to be reported "...at start of test and on days 7, 14, 21, and 28 of the test" (OPPTS 850.6200, Earthworm Subchronic Toxicity Test, US EPA, Prevention, Pesticides and Toxic Substances (7104), EPA 712-C-96-167, April 1996, p. 10, item (vii)).

5. The pH was adjusted with calcium carbonate to 5.6. The amount of calcium carbonate used was not reported. Guideline regulations specify that up to 2 percent of pulverized calcium carbonate may be added to adjust the pH to 6.5 ± 0.5 (OPPTS 850.6200, Earthworm Subchronic Toxicity Test, US EPA, Prevention, Pesticides and Toxic Substances (7104), EPA 712-C-96-167, April 1996, p. 7).

6. The reported concentrations of the test substance are assumed to be the initial concentrations at the beginning of the study. Guideline regulations specify that "the concentration of the test substance in artificial soil should be measured at a minimum in each chamber at the beginning (zero-hour, before earthworms are added) and every 7 days thereafter" (OPPTS 850.6200, Earthworm Subchronic Toxicity Test, US EPA,

Prevention, Pesticides and Toxic Substances (7104), EPA 712-C-96-167, April 1996, p. 5, item (A)).

7. Worms were counted on days 0, 7 and 14 and weighed on days 0 and 14. Guideline regulations specify that "each test and control chamber should be checked for dead or affected earthworms and observations recorded 7, 14, 21, and 28 days after the beginning of the test..." (OPPTS 850.6200, Earthworm Subchronic Toxicity Test, US EPA, Prevention, Pesticides and Toxic Substances (7104), EPA 712-C-96-167, April 1996, p. 4, Test Results, item (iii)).

8. The relative humidity was not reported. The guidelines specify that "relative humidity should be maintained above 85%" (OPPTS 850.6200, Earthworm Subchronic Toxicity Test, US EPA, Prevention, Pesticides and Toxic Substances (7104), EPA 712-C-96-167, April 1996, p. 7).

9. The light intensity was 500 lux. The guidelines specify that light intensity should be about 400 lx measured at the artificial soil surface" (OPPTS 850.6200, Earthworm Subchronic Toxicity Test, US EPA, Prevention, Pesticides and Toxic Substances (7104), EPA 712-C-96-167, April 1996, p. 8).

10. SUBMISSION PURPOSE: This study was submitted to provide data on the toxicity of chlorophenyl urea to earthworms for the purpose of chemical registration.

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species: *Eisenia fetida* **andrei (Bouche)**	*Eisenia foetida*
Weight: 300-600 mg	365-458 mg
Age: Adult	Age not specified.

Guideline Criteria	Reported Information
Source:	Huntingdon Life Sciences stock.

B. Test System

Guideline Criteria	Reported Information
Test Container: **Glass canning jars (1 pint capacity) or equivalent**	1 L glass containers covered with perforated plastic.
Artificial Soil Medium: **Dry weight mixture of:** **68% No. 70 mesh silica sand,** **20% kaolin clay,** **10% sphagnum peat moss,** **2% calcium carbonate**	70% industrial sand 20% kaolin clay 10% sphagnum peat Added for pH adjustment (percentage not specified)
Weight of Soil: **270 g (wet soil)**	739 g
Moisture Content of Soil: **35%**	35% (test initiation) 32-33% (test termination)
Temperature: **22 ± 2°C**	21-22°C
Relative Humidity: **≥85%**	Not reported
Light Intensity: **400 lux**	500 lux
Photoperiod: **Continuous**	Continuous
pH: **6.5 ± 0.5**	5.6 (initiation)

C. Test Design

Guideline Criteria	Reported Information
Dose range: **ratio of 1.5 or 2.0 mg/kg**	Approximately 1.8 mg/kg ratio
Doses: **at least 5**	95, 171, 309, 556, and 1000 mg/kg
Controls: **at least 1**	1 control (acetone)
Replicates per Dose: **3**	4
Number of Worms per Replicate: **10**	10
Test duration: **at least 28 days**	14 days
Observations made every 7 days after test initiation for dead or affected worms?	Mortalities was observed on days 7 and 14. The behavior and pathological signs were observed daily for worms on soil surface. Weights were recorded prior to treatment and on day 14.
Maximum labeled rate:	Not reported.

12. <u>REPORTED RESULTS</u>:

Guideline Criteria		Reported Information
Initial and 7-, 14-, 21-, and 28-day:	**worm weight reported?**	Initial and day 14 worm weights were reported.
	temperature and pH reported?	Temperature data not reported (range was provided); initial pH value was reported.
	chemical concentrations reported?	Mean measured concentrations were not reported.

Guideline Criteria	Reported Information
Raw data included?	Raw data were reported.

Dose Response

Nominal Concentration in Soil (mg/kg)	Mean Weight (mg) at Day:				Weight Decrease (%)	# of Dead Worms at Day:				Mortality (%)
	0	7[NR]	14	28*		0	7	14	28*	
Control	412	-	443	-	-8	0	0	0	-	0
95	406	-	418	-	-3	0	0	0	-	0
171	409	-	420	-	-3	0	0	0	-	0
309	411	-	365	-	11	0	0	1	-	2.5
556	408	-	294	-	28	0	2	33	-	88
1000	410	-	---	-	---	0	38	2	-	100

NR = not reported
--- Mortality was 100%.
* the test duration was 14 days, therefore, no results exist for day 28.

Statistical results:

Statistical Method: The percent change in treatment group weights were compared to the control. The LC_{50} was calculated using a logistic model (Berkson 1944) and the 95% confidence limits were estimated by the likelihood ratio method (Williams 1986). The NOEC and LOEC were visually determined using the weight and mortality data.

LC_{50}: 454 mg/kg 95% C.I.:411-496 mg/kg
NOEC:171 mg/kg Probit Slope: N/A
LOEC: 309 mg/kg

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The NOEC and LOEC were determined by visually inspecting the mortality and body weight data. The LC_{50} was estimated using the probit method via TOXANAL statistical software.

LC_{50}: 447 mg/kg	95% C.I.:407-485 mg/kg
NOEC:171 mg/kg	Probit Slope: 12.2 (8.4-16.0)
LOEC: 309 mg/kg	

14. REVIEWER'S COMMENTS:

The reviewer's conclusions regarding the NOEC and LOEC values were identical to the study author's. However, the reviewer's LC_{50} was slightly lower and the 95% confidence interval was narrower. As a result, the reviewer's LC_{50} estimate is reported in the Conclusions section. The difference between the two estimates is due to the different methods used to derive these values.

In order to validate the test system, the reference toxicant chloroacetamide was tested November 3-17, 1998. The LC_{50} for chloroacetamide was 53.1 mg/kg with 95% confidence interval of 48.1-59.3 mg/kg. The results of the reference toxicant test confirmed the validity of the definitive test.

15. REFERENCES:

Berkson, J. (1944) Application of the logistic function to bio-assay. *J. Amer. Statist. Assoc.* 39, 357-365.

Williams, D.A. (1986) Interval Estimation of the median Lethal Dose. Biometrics, 42, 641-645.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	2.376378E-02	447.2494	404.0751	496.8728

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
22	9.615157E-02	1	.9999895

SLOPE = 12.19378
95 PERCENT CONFIDENCE LIMITS = 8.412691 AND 15.97486

LC50 = 447.422
95 PERCENT CONFIDENCE LIMITS = 407.3303 AND 485.0663

LC10 = 352.0189
95 PERCENT CONFIDENCE LIMITS = 298.2545 AND 389.6717